ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.323

Volume 124, pp. 323–332 *April–June 2013*

Studies of North American macrofungi, 1. Validation of *Lactarius rubidus* comb. nov. and *Leccinellum quercophilum* sp. nov.

Michael Kuo,[1]* Andrew S. Methven,[2]
Andrew M. Minnis,[3] & Roy E. Halling[4]

[1]*Department of English &* [2]*Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston IL 61920 USA*
[3]*Center for Forest Mycology Research, Northern Research Station, USDA-U.S. Forest Service, One Gifford Pinchot Dr., Madison WI 53726 USA*
[4]*Institute of Systematic Botany, The New York Botanical Garden, 2900 Southern Boulevard, Bronx NY 10458 USA*
* Correspondence to: *michael@mushroomexpert.com*

Abstract — Two mycorrhizal taxa are described: *Lactarius rubidus* from the west coast and *Leccinellum quercophilum* from eastern North America. *Lactarius rubidus* is a new combination for *Lactarius fragilis* var. *rubidus,* here validated after a previous invalid publication. *Leccinellum quercophilum* is a new species. Detailed macro- and microscopic descriptions, ITS and LSU sequences, illustrations of microscopic features, and plates are presented for each species; a type study of *Lactarius fragilis* var. *rubidus* is also provided.

Key words — *Boletaceae, Boletales, Russulaceae, Russulales*, taxonomy

Introduction

The North American macrofungi known as *Lactarius fragilis* var. *rubidus* and *Leccinum griseum* (also sometimes known as *Leccinum carpini*) are commonly collected fungi for which currently applied names are unsatisfactory. The west coast fungus *Lactarius fragilis* var. *rubidus,* which differs substantially from the eastern North American type variety in morphology, ecology, and geographic distribution, merits taxonomic rank as a species. *Leccinum griseum* is a European fungus clearly distinct from the North American entity frequently bearing its name in herbaria. Here we describe *Leccinellum quercophilum*, a North American bolete sometimes identified as *Leccinum griseum,* as a new species and validate the combination *Lactarius rubidus* to apply to *Lactarius fragilis* var. *rubidus*.

Materials & methods

Both fresh basidiomes and herbarium specimens were studied. Colors were recorded and codified following Kornerup & Wanscher (1967), or the Online Auction Color Chart (www.onlineauctioncolorchart.com — abbreviated "OAC" herein). Microscopic features were studied using hand sections of fresh material, and of dried specimens rehydrated in water after immersion in 90% alcohol. Sections were mounted in 2% KOH and Melzer's reagent, and viewed using an Olympus BX51 microscope. Specimens are deposited in herbaria and cited according to Thiers (2012).

DNA from each taxon was extracted from small hymenium-bearing pieces of dried basidiocarp following the methods of Lindner & Banik (2009). Protocols for ITS amplification, sequencing, sequence editing, and other related procedures followed Lorch et al. (2013) with the following modifications: denaturing for 40 s, annealing at 53 °C for 40 s, and extension for 90 s in the 30 PCR cycles. LSU sequences were obtained with the same protocols using the primers LROR (Moncalvo et al. 2000) and either LR5 or LR7 (Vilgalys & Hester 1990). Newly generated DNA sequences were deposited in GenBank and compared with available sequences via GenBank BLAST searches.

Taxonomy

Lactarius rubidus (Hesler & A.H. Sm.) Methven, **comb. & stat. nov.** Plate 1

MycoBank MB803100

≡ *Lactarius fragilis* var. *rubidus* Hesler & A.H. Sm., N. Amer. Sp. *Lactarius*: 505. 1979.

"*Lactarius rubidus*" Methven, *Agaricales* (Gilled Fungi) of California, 10, II: 67. 1997, nom. inval. [provisional name].

Pileus 2–8 cm broad, convex to plane, disc shallowly depressed, occasionally umbonate; margin incurved to decurved, glabrous; surface glabrous, dry to moist, rugose to rugulose, azonate, light brown (6D7-5) to brown (7D8-5), or reddish brown (8D8-6), negative with the application of 15% KOH; context up to 5 mm thick at the disc, light orange (5A4-2), unstaining on exposure; odor of maple syrup or fenugreek when dried; taste not distinctive. Lamellae adnate to subdecurrent, close to distant, narrow, rarely forked, light orange (5A4-2, 6A4-3), not marginate, unstaining where cut. Stipe 2–7 cm × 4–10(–15) mm, terete, equal; surface glabrous, dry, not scrobiculate, brownish orange (6C7-5) to light brown (7D7-5); white to light orange tomentum at the base; context hollow, concolorous with the pileal context, unstaining on exposure. Latex whey-like, not copious, unstaining, unchanging, taste not distinctive.

Basidiospores white to pale yellow (4A3-2) in mass, 6–8(–8.5) × 6–7.5 µm, globose to subglobose; amyloid ornamentation a broken to partial or nearly complete reticulum 0.5–1 µm high. Basidia 35–55 × 7.5–12.5 µm, tetrasterigmate. Cheilocystidia 25–35 × 6–9 µm, clavate. Macrocystidia not observed. Pileipellis an epithelium composed of clavate to vesiculose cells in short chains, tangled in age, dry. Pileus trama heteromerous, sphaerocysts in rosettes, lactiferous hyphae inconspicuous. Stipitipellis a simple cutis with

Plate 1: *Lactarius rubidus*, M. Kuo 01131106 (NY). Basidiocarps (top), basidiospores (middle), and pileipellis (bottom). Scale bars = 10 µm. [For additional illustrations see Hesler & Smith (1979: Fig. 247, basidiospores) and Methven (1997: Pl. 2, basidiocarps).]

scattered projecting hyphal tips, stipe cortex heteromerous, sphaerocysts in rosettes.

Ecology & distribution — Scattered, gregarious, or caespitose in duff, in coastal coniferous-deciduous forests in apparent association with *Quercus agrifolia, Notholithocarpus densiflorus,* and *Pseudotsuga menziesii.* Common. September through February. California, Oregon, and Washington.

Collections examined — **UNITED STATES. California**: Marin County, 10 Dec 1982, ASM 2261 (EIU); 19 Dec 1984, ASM 3523 (EIU); 27 Dec 1984, ASM 3632

(EIU). Mendocino County, 30 Apr 1983, CM Bern, ASM 2456 (EIU); 13 Dec 1990, ASM 6523 (EIU). San Mateo County, 17 Dec 1984, ASM 3600 (EIU); 12 Jan 1985, H Saylor 2297 (EIU); 13 Jan 2005, M Kuo 01130522 (NY); 13 Jan 2011, M Kuo 01131106 (NY, GenBank ITS KC691205, LSU KC691206). Sonoma County, 02 Feb 2003, M Kuo 02200309 (NY). **Oregon**: Polk County, 14 Nov 1970, AH Smith 79939 (MICH 23242, paratype); 14 Nov 1970, AH Smith 79942 (MICH 11133, holotype).

Comments — *Lactarius rubidus* is characterized by the reddish brown, azonate, dry to moist pileus, whey-like latex, the maple syrup or fenugreek odor when dried, and globose basidiospores with a broken to partial or nearly complete amyloid reticulum. *Lactarius fragilis* (Burl.) Hesler & A.H. Sm. var. *fragilis,* known from eastern North America, differs in having a snuff brown to burnt umber pileus, subdistant yellowish lamellae, and globose basidiospores with heavier deposits of amyloid ornamentation that form a partial to complete reticulum. *Lactarius rubidus* has frequently been misidentified as *L. camphoratus* (Bull.) Fr. However, *L. camphoratus* is a European and eastern North American species featuring a red brown pileus, close to crowded pale pinkish cinnamon lamellae, milk-white latex, and broadly ellipsoid basidiospores with amyloid ornamentation of isolated spines and nodulose ridges forming at most a broken reticulum. *Lactarius rubidus* is a popular edible commonly called the "candy cap" by collectors (Kuo 2007). Wood et al. (2012) recently determined quabalactone III to be the source of the maple syrup odor of *L. rubidus*.

ITS sequence comparisons via GenBank BLAST searches indicate *L. rubidus* is not conspecific with European and North American *L. camphoratus*. *Lactarius* cf. *rubidus* (GenBank DQ822820.1 from a basidiocarp and GU180303.1 from a *Pinus muricata* root tip; Peay et al. 2007) and unidentified samples GenBank AF323116.1 from a *Pinus muricata* root (Wurzberger et al. 2001) and DQ273391.1 from a *Notholithocarpus densiflorus* root (Bergemann & Garbelotto 2006), all from California, appear to represent *L. rubidus* at 99% sequence identity.

In Methven (1997: 67), the combination "*Lactarius rubidus* (Hesler & A.H. Sm.) Methven" appeared with a citation of its forthcoming publication as "Mycotaxon (in press). 1998" but was not validly published since it was merely proposed in anticipation of future acceptance of this taxon at the species rank (ICN Art. 36.1, McNeill et al. 2012). The subsequent Mycotaxon publication did not occur, and the combination *Lactarius rubidus* has not been validly published elsewhere since. A record for "*Lactarius rubidus* Arora 1991" appears in Index Fungorum (www.indexfungorum.org) but no further information is provided; a similar record appears in the MycoBank database (www.mycobank.org), again without any supporting publication information. David Arora's (1991) field guide, All That the Rain Promises and More: a Hip Pocket Guide to Western Mushrooms is the only plausible basis we have been able to discover for these website entries. Since Arora treats the species as "*Lactarius fragilis*," listing

"other names" as "*Lactarius fragilis* var. *rubidus, L. rubidus*," without citing taxon authorities or providing full and direct references to any of the names as required for publishing a new combination, his book cannot be considered a valid publication of *Lactarius rubidus*. We validate the combination here.

Study of the Holotype — The holotype of *Lactarius fragilis* var. *rubidus* is held in the University of Michigan Herbarium (MICH 11133) and was collected by A.H. Smith (79942) on "rotten wood" in the Van Duzer Corridor, Polk County, OR; it consists of several well preserved basidiomes with a strong odor of fenugreek. Basidiospores 6.5–8.5 × 6–7.5 µm, globose to subglobose, ornamentation a partial to more or less complete reticulum 0.5–1 µm high, with scattered free ends and rare isolated elements, plage conspicuous and lacking amyloid debris. Basidia 35–50 × 7.5–12.5 µm, clavate, tetrasterigmate. Cheilocystidia not clearly differentiated. Macrocystidia not observed. Pileipellis an epithelium composed of vesiculose or irregularly shaped inflated cells in short chains, at times aggregated into mounds, especially in age. Pileus Trama heteromerous. Stipitipellis a dry, simple cutis of interwoven hyphae with scattered, projecting, pyriform to subfusiform hyphal tips 25–35 × 7.5–15 µm.

Leccinellum quercophilum M. Kuo, **sp. nov.** Plate 2

MycoBank MB802407

Differs from *Leccinum griseum* by its bluish green staining on the pileus and stipe, its creamy whitish tubes, its context staining gray when sliced, and its association with *Quercus* in eastern North America.

Type: United States, Illinois, Coles County, Charleston, under *Quercus alba* on a lawn, 12 July 2008, coll. M Kuo 07120801 (**Holotype**, NY; GenBank ITS KC691207, LSU KC691208).

Etymology: from a combination of the Latin *Quercus* = oak, and the Greek φιλος = friend; "oak-loving."

Pileus 3–9 cm broad; convex; dry; glabrous; dull orangish brown (OAC 749) to medium yellow-brown (OAC 707, 715); rugulose-pitted when young, becoming conspicuously areolate; without an overlapping sterile margin; discoloring bluish green marginally with age; surface negative with the application of ammonia or $FeSO_4$, negative to yellowish with 15% KOH. Hymenophore tubulose; depressed at the stipe. Tubes 1–2 cm deep, creamy whitish (contrasting with the pore surface), discoloring slowly blackish when sliced; pores 1–3 per mm at maturity, round becoming angular with maturity, whitish or medium grayish brown (OAC 736) when young, becoming yellowish brown to brownish; discoloring greenish in places with age; bruising slowly dark brown, with or without a bluish stage. Stipe 5–9 cm x 8–20 mm; equal, or tapered slightly to apex, or slightly ventricose; dry; whitish underneath fine, tiny scabers that are whitish apically and brown below; the scabers arranged in vague longitudinal

lines, darkening somewhat with age but not blackening; bruising or staining greenish to bluish basally; basal mycelium whitish. Context whitish, staining grayish to gray within 15–30 minutes of being sliced, with or without a faintly pinkish stage; sometimes staining bluish in the stem base when sliced; pinkish to negative with the application of ammonia, negative to greenish with $FeSO_4$, gray to greenish gray with 15% KOH. Odor and taste not distinctive.

Basidiospores 15–18(–28) × 5–7.5 µm; fusiform; ochraceous in 2% KOH; inamyloid; aseptate; smooth. Basidia 25–30 × 9–12 µm; clavate; tetrasterigmate. Pleurocystidia 25–40 × 8–12 µm; occasional or frequent; fusoid-ventricose; thin-walled; smooth; hyaline to ochraceous or golden in 2% KOH. Cheilocystidia 35–50 × 5–10 µm; fusoid-ventricose to mucronate, subclavate, or irregular; thin-walled; smooth or roughened; ochraceous to golden in 2% KOH. Caulocystidia 30–50 × 10–15 µm; mostly fusoid-ventricose but occasionally ventricose-rostrate, or lageniform; apices occasionally mucronate; thin-walled; smooth; hyaline to ochraceous in 2% KOH. Pileipellis a trichodermium of septate hyphae; hyaline to ochraceous or brownish in 2% KOH; terminal elements subglobose to clavate or irregular, 25–50 µm wide.

Ecology & distribution — Scattered to gregarious under oaks; appearing in hardwood forests and in urban settings (lawns, cemeteries, etc.); summer and early fall; currently known from Illinois and Michigan.

Additional collections examined — **UNITED STATES. Illinois**: Coles County, 20 Jun 2010, M Kuo 06201003 (NY). **Michigan**: Livingston County, Aug 1972, AH Smith 84011 (MICH 35770, as *Leccinum griseum*); Washtenaw County, 16 Sep 1940, AH Smith 15410 (MICH 35774, as *Leccinum griseum*).

Comments — *Leccinellum quercophilum* can be identified by its association with oaks, the dull brown or yellowish brown rugulose-pitted cap that becomes conspicuously areolate, the fine brown scabers, the green and blue staining, and the trichoderm pileipellis. It has been thought to represent the European species *Leccinum griseum* (Quél.) Singer (≡*Gyroporus griseus* Quél.) by various North American authors, including Smith & Thiers (1971: 214). However, the name *Leccinum griseum* is problematic (Lannoy & Estades 1995; den Bakker & Noordeloos 2005). Regardless of its correct name, this European species, sometimes listed as *Leccinum carpini* (R. Schulz) D.A. Reid (Lannoy & Estades 1995) or *Leccinum pseudoscabrum* (Kallenb.) Šutara (den Bakker & Noordeloos 2005), is primarily associated with *Carpinus* and does not stain blue or green (except very rarely in the base of the stem). Also, the European species has yellowish tubes when young and flesh that stains deep violet when sliced (Lannoy & Estades 1995; den Bakker & Noordeloos 2005)—a difference noted by Smith & Thiers (1971). On the basis of these morphological differences, we recognize the North American species as new and have selected an epithet reflecting its association with oaks.

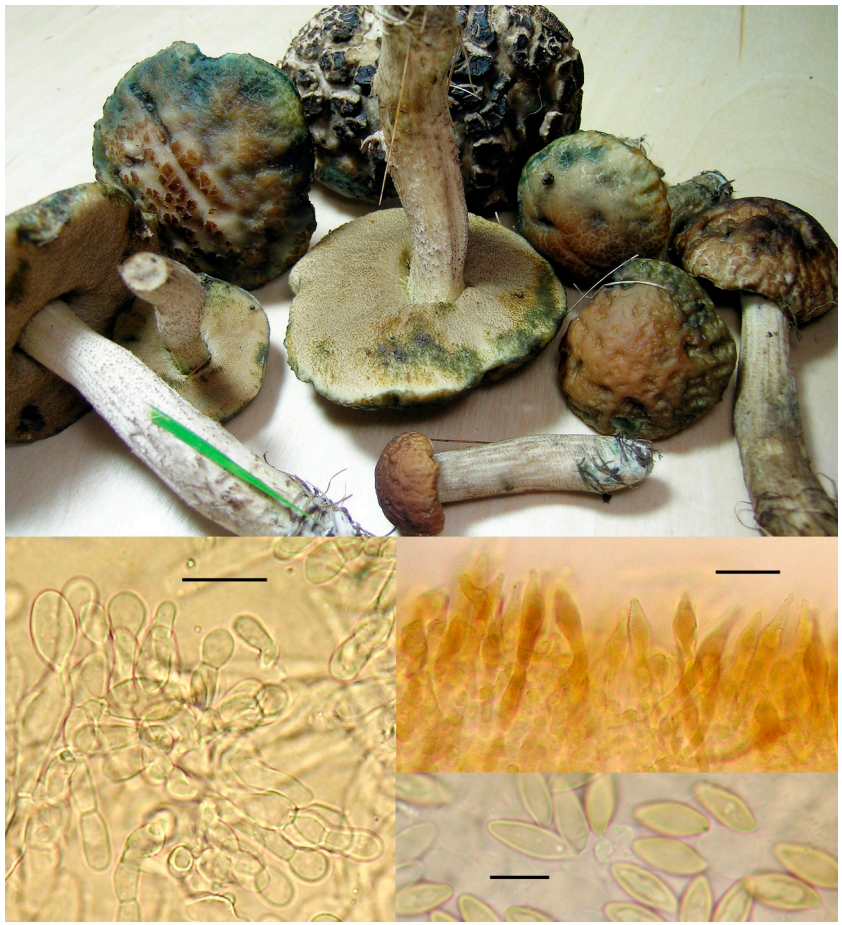

Plate 2: *Leccinellum quercophilum*, M. Kuo 07120801 (NY; holotype). Basidiocarps (top), pileipellis elements (bottom left), cheilocystidia (middle right), and basidiospores (bottom right). Scale bars = 10 µm.

Leccinellum Bresinsky & Manfr. Binder was erected in 2003 (Bresinsky & Besl 2003) to accommodate *Leccinum* species with a yellow hymenophore and a trichoderm-like pileipellis. *Boletus nigrescens* Richon & Roze (nom. illegit.; ≡ *Leccinellum nigrescens* (Singer) Bresinsky & Manfr. Binder) was designated as the type of the genus. Bresinsky & Besl (2003) included *Leccinum carpini* and *L. griseum* among eight existing species they accepted in *Leccinellum*, but the nomenclature and taxonomy warrant review. Although the hymenophore of *Leccinellum quercophilum* is not yellow, it falls within *Leccinellum* as defined in Bresinsky & Besl (2003) on the basis of its pileipellis.

GenBank BLAST searches with an ITS sequence of *Leccinellum quercophilum* reveal that the closest matches include *Leccinum carpini* [AF454588.1 (The Netherlands)], *Leccinum crocipodium* (Letell.) Watling [AF454589.1 (Belgium), JN021053.1 (France), JF908325.1 unpublished (Italy), and AF454590.1 (The Netherlands)], and *Leccinum talamancae* Halling et al. [AY544779.1 (Costa Rica)] as labeled and numbered in GenBank with published citations (den Bakker et al. 2004a,b; Dentinger et al. 2011). The >3% difference in sequence identity supports *Leccinellum quercophilum* as a distinct species. Morphological differences also separate these taxa. As noted above, the European species associated with *Carpinus* has yellowish young tubes, flesh that turns violet when sliced, and does not stain blue or green; *Leccinum crocipodium* has a yellow hymenophore and does not stain green (den Bakker & Noordeloos 2005); *Leccinum talamancae* is less areolate and features flesh that turns slowly pink to reddish orange in the pileus and stipe apex when exposed (Halling 1999). Our own additional unpublished alignments and phylogenetic analyses, along with the ITS tree in Lebel et al. (2012a,b), support generic placement in *Leccinellum*. Further research will determine whether *Leccinellum* as presently circumscribed is or is not supported phylogenetically.

Acknowledgments

We are grateful to Timothy J. Baroni and Patrick R. Leacock for serving as pre-submission reviewers and for their helpful comments on the manuscript; Darvin DeShazer also provided helpful commentary. We thank the University of Michigan Herbarium (MICH) for kindly lending specimens, and the William and Linda Steere Herbarium of the New York Botanical Garden (NY) for processing deposits. We also thank Henk den Bakker, Dana Ringuette, Barbara Thiers, Jean Toothman, and Sue Yocum for invaluable assistance in various aspects of this study. The U.S. Forest Service, Northern Research Station, Center for Forest Mycology Research provided funds for DNA sequencing. We thank Kyah Norton of the CFMR for providing expert technical support. Support to REH from the National Science Foundation, via grants DEB #9972018, DEB #0414665, and DEB #1020421, is gratefully acknowledged.

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